2C DNA CONTENT VALUES IN *AMARANTHUS* (AMARANTHACEAE)

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ABSTRACT

Feulgen densitometry was used to measure 2C DNA content values from 15 accessions of 12 species of *Amaranthus* representing all subgenera. Species showed a 3-fold variation in range from 0.89 pg (*Amaranthus viridis*) to 2.73 pg (*Amaranthus tricolor*). Values are compared to previous reports and taxonomic implications of DNA content values are discussed.

RESUMEN

Se usó la densitometría de Feulgen para medir los valores del contenido de 2C DNA de 15 accesiones de 12 especies de *Amaranthus* que representan todos los subgéneros. Las especies mostraron una variación de 3-veces variando de 0.89 pg (*Amaranthus viridis*) a 2.73 pg (*Amaranthus tricolor*). Se comparan los valores con informes previos y se discuten las implicaciones taxonómicas de los valores de contenido de DNA.

INTRODUCTION

Amaranthus L. (Amaranthaceae) is a genus of about 70 species of monoecious or dioecious annuals of world-wide distribution. The genus is economically important, including the cultivated grain amaranths, potherbs, and ornamentals as well as a suite of agricultural and range weeds. Traditionally the genus has been divided into two subgenera, subg. *Amaranthus* (the monoecious amaranths) and *Acnida* (dioecious amaranths) based on breeding system (Robertson 1981). The most recent classification system divides *Amaranthus* into three subgenera: *Amaranthus* (with 12 species), *Acnida* (with 9 species), and *Albersia* (with 49 species) based on breeding system and characteristics of the fruit and flowers (Mosyakin & Robertson 1996).

Chromosome counts of the Amaranthaceae reveal a base count of x = 8 or x = 9 (Turner 1994). *Amaranthus* itself appears to be paleopolyploid with counts of $n = 16$ or $n = 17$ (Grant 1959a; Grant 1959c; Greizerstein & Poggio 1992; Greizerstein & Poggio 1994), and genomes behave as diploids during meiosis with the exception of *Amaranthus dubius*, an allotetraploid with $n = 32$ between *A. spinosus* and an unknown species (Grant 1959b; Greizerstein & Poggio 1992). Cytogenetic research demonstrated that variation between $n = 16$ and $n = 17$ can occur within individuals of a single population, the extra chromosome in individuals with counts of $n = 17$ is derived via primary trisomy from $n = 16$ (Pal et al. 1982; Griezerstein & Poggio 1994). In addition to chromosome number, karyotypic work has demonstrated that *Amaranthus* chromosomes are of small size (Grant 1959a; Grant 1959c; Griezerstein & Poggio 1994); possess a single NOR locus (Griezerstein & Poggio 1994); and demonstrate a wide variety of C banding patterns putatively due to differences in heterochromatin content (Griezerstein & Poggio 1994).

Aside from chromosome counts, very little research has been directed towards analysis of DNA content in *Amaranthus*. To date, 2C DNA content in picograms has been recorded for 14 species in *Amaranthus* (Griezerstein & Poggio 1994; Bennett et al. 2000; Jeschke et al. 2003; Rayburn et al. 2005). Taxonomic

sampling of the DNA content studies is highly skewed. Of the 14 reported counts, nine reports come from the weedy and cultivated species of subgenus *Amaranthus* (which contains an estimated 12 species), two reports from subg. *Acnida* (which contains nine species), and three reports from subg. *Albersia* (which contains 49 species). However, given the wide range of previously reported DNA content values, it would be prudent to expand sampling of subg. *Acnida* and *Albersia*.

The objectives of this study were therefore twofold: 1) to obtain estimates of DNA content from a wide variety of species of *Amaranthus* including representatives of all three subgenera using an image analysis densitometry technique; and, 2) to explore possible taxonomic significance of DNA content variation.

MATERIALS AND METHODS

Seed from fifteen accessions and twelve species of *Amaranthus* were donated by the USDA North Central Regional Plant Introduction States (NCRPIS). Known 2C DNA values for *Amaranthus* were obtained from the database at Kew Gardens (data.kew.org/cvalues/), and *Sorghum bicolor* Pioneer cultivar 8695 was chosen as the reference standard based on its 2C DNA content of 1.74 pg (Johnston et al. 1999) which compared favorably to the range of values (0.96–2.46 pg) reported in the Kew database.

Amaranth seeds were stratified at 4°C for one month and plants grown in a greenhouse. Roots were fixed in 3:1 ethanol: glacial acetic acid solution and stored in 70% ethanol at 4°C. Fixed root material was hydrolyzed in 5N HCl for 30 minutes and stained in freshly prepared and charcoal-filtered Fuelgen stain for three hours, and destained for 15 minutes in distilled water. Fuelgen stained roots tips were softened 45 minutes in 2% w/v cellulysin enzyme in 0.001 M EDTA pH 5.6 solution, replaced with 45% acetic acid, squashed, cryofixed in liquid nitrogen, and dried overnight before adding coverslips.

Chromosome squashes were photographed on an Olympus BX50 Light Microscope using a Nikon UR-E6 Coolpix MDC Lens attached to a Nikon Coolpix 5000 digital camera. Twenty to thirty images of the stained nuclei per specimen were uploaded, analyzed using a wavelength of 560 nm (Hardie et al. 2002), and integrated optical density (I.O.D.) values were calculated using Image Pro Plus 5.1 (Media Cybernetics, Inc., Maryland).

IOD values for nuclei were pooled after discarding the top and bottom 5% of values if an ANOVA indicated no difference between the means from different images (Hardie et al., 2002), and mean IOD values were calculated from the pooled data. A coefficient of variation (COV) was calculated (mean / stdev.) and the IOD data was considered usable only if the COV was less than 10% (Vilhar et al. 2001; Hardie et al. 2002). Absolute DNA contents in picograms for all *Amaranthus* populations were determined comparing the mean IOD values to the mean IOD for *S. bicolor* standard that was processed in the same stain batch as the *Amaranthus* spp using the following calculation: $[{}^{\text{Amaranth IOD}}/_{\text{Sorghum IOD}}]*1.74$ pg

RESULTS

Mean 2C DNA content values are reported in Table 1. Among the fifteen accessions sampled we found 3-fold variation in 2C DNA contents, ranging from a low of 0.89 pg in *A. viridis* to a high of 2.73 in *A. tricolor*. The two subspecies of *A. graecizans* had a 1.1-fold difference in values (1.33 and 1.46). The two accessions of *A. arenicola* had 1.31 fold difference in content values (1.15 and 1.5). The two accessions of *A. blitum* had a 1.14-fold range in content values (1.06 and 1.21). Subgenera *Acnida* and *Albersia* showed a 1.14 and 1.07-fold increase in DNA content values over values found in subgenus *Amaranthus*. Within subg. *Albersia*, section *Pyxidium* showed a 1.8-fold increase in DNA values over sections *Blitopsis* and *Pentamorion*.

DISCUSSION

We report 2C DNA content values from 15 accessions of 12 species (Table 1), and provide reports for eight species and two sections (*Blitopsis* and *Pentamorion*) that had previously been unreported in the literature (Table 2), expanding the sampling from *Amaranthus* to 23 species. Our values compare favorably to previously reported values (Table 2).

TABLE 1. List of sampled accessions, species, and 2C DNA content values.

Taxon	Location	Accession	2 C
Subgenus *Amaranthus*			1.23
A. caudatus	Peru	PI 490440	1.32
A. dubius	Panama	Ames 25792	1.23
A. powellii bouchonii	Germany	PI 572261	1.13
Subgenus *Acnida*			1.40
A. arenicola	Kansas	PI 607459	1.51
A. arenicola	Kansas	PI 599671	1.15
A. greggii	Texas	PI 632240	1.54
Subgenus *Albersia*			1.32
Sect. *Blitopsis*			1.02
A. acutilobus	Germany	PI 633578	1.22
A. blitum	India	PI 608661	1.06
A. blitum	India	PI 610262	1.21
A. viridis	Maldives	PI 536439	0.89
Sect. *Pyxidium*			1.84
A. tricolor	Pennsylvania	PI 599683	2.73
A. graecizans silvestris	India	Ames 24671	1.46
A. graecizans thellungianus	Mauritius	PI 549157	1.33
Sect. *Pentamorion*			1.01
A. crassipes	Cuba	Ames 10339	0.97
A. standleyanus	Argentina	PI 61380	1.02

Values from subg. *Amaranthus* range from 1.04 pg in *A. hybridus* to 1.9 pg in *A. spinosus* with a composite average (our values as well as all other reported values) of 1.29 pg (Table 2). Two of the reported values, *A. retroflexus* 1.7 pg and *A. spinosus* 1.9 pg, are suspiciously high (1.31 and 1.4-fold higher than the average) compared to other reported values for these species and for other member of subg. *Amaranthus* (Table 2). If these two values are removed, the range of values for the subgenus decreases to 1.04–1.35 pg, with a composite average of 1.23 pg which matches our values closely (Table 2).

Several DNA content values from subg. *Amaranthus* are noteworthy. The 1.23 pg DNA content for the allotetraploid *A. dubius* is particularly intriguing (Table 1). The most likely 2C DNA content value of the known parental species, the diploid *A. spinosus*, is 1.10 pg (Table 2). If these values for *A. dubius* and *A. spinosus* are correct, then either the DNA content of *A. dubius* has undergone a major contraction following the polyploidization event, or the unknown parent has a very small genome size. The reported values for *A. hybridus* and *A. quitensis* (a South American form of *A. hybridus*) range from 1.04 to 1.30 pg, with an average of 1.18 pg. This species is a wide-ranging, cosmopolitan weed that originated in the Americas. The range in values may represent geographic or taxonomic patterns of distribution that should be better examined.

Reported values from subg. *Acnida* (the dioecious amaranths) represent a 1.62-fold range from 0.95 to 1.54 pg in DNA content (Table 2). This subgenus is closely related to species of Subg. *Amaranthus* as determined by crossing data (Murray 1940; Trucco 2006). The large range of reported values may represent considerable changes in DNA content following the evolution of dioecy. There is a 1.3 fold difference in 2C DNA content values for *A. arenicola*. The accessions were collected from the Arkansas River in Kansas two years apart by different collectors. *Amaranthus arenicola* is a wild species endemic to the area, and has received little study. These data indicate the possibility that the area may be a center of diversity for the species and further research into the species is warranted. The reported DNA content value for *A. palmeri*

Table 2. DNA content values from current research and literature review.

	Our Value	Literature
Subgenus *Amaranthus*		
A. caudatus	1.32	1.30[1]
		1.35[2]
		1.26[5]
A. cruentus		1.30[1]
		1.26[2]
A. dubius	1.23	
A. hybridus		1.30[1]
		1.04[2]
A. hypochondriacus		1.30[1]
		1.26[2]
A. powellii powellii		1.20[1]
		1.14[3]
A. powellii bouchonii	1.13	1.20[4]
A. quitensis		1.20[1]
A. retroflexus		1.70[1]
		1.23[3]
A. spinosus		1.90[1]
		1.10[3]
Average values	1.23	1.30
Composite average	1.29	
Subgenus *Acnida*		
A. arenicola	1.51	
	1.15	
A. greggii	1.54	
A. palmeri		0.95[3]
A. tuberculatus		1.34[3]
Average values	1.40	1.14
Composite average	1.30	
Subgenus *Albersia*		
Sect. *Blitopsis*		
A. acutilobus	1.22	
A. blitum	1.06	
A. blitum	1.21	
A. viridis	0.89	
Average values	1.02	na
Composite average	1.02	
Sect. *Pyxidium*		
A. albus		1.18[4]
A. blitoides		1.15[4]
A. tricolor	2.73	1.80[1]
	2.46[5]	
A. graecizans silvestris	1.46	
A. graecizans thellungianus	1.33	
Average values	1.84	1.65
Composite average	1.73	
Sect. *Pentamorion*		
A. crassipes	0.97	
A. standleyanus	1.02	
Average values	1.01	na
Composite average	1.01	

[1]Bennett, Bhandol, & Leitch 2000
[2]Greizerstein & Poggio 1994
[3]Jeschke, Tranel, & Rayburn 2003
[4]Rayburn et al. 2005
[5]RGB Kew Gardens DNA C-Values Database

(0.95 pg, Table 2) is very low compared to other dioecious waterhemps, but is in-line with recorded values for *A. spinosus* (1.10 pg, Table 2). These DNA content values support Wassom and Tranel's (2005) AFLP data, which placed *A. palmeri* in a clade with *A. spinosus* rather than with other dioecious amaranths.

We provide DNA content data for six new species and eight accessions from subg. *Albersia* (Table 2). *Albersia* is the largest subgenus of *Amaranthus*, although most of the species are poorly known (Mosyakin and Robertson 1996). DNA content values in the subgenus ranged from the smallest value (*A. viridis* with 0.89 pg) to the largest (*A. tricolor* with 2.73 pg) in the study (Table 2) indicating that there is a great deal of variation in DNA content values within the subgenus. Values within sections *Blitopsis* (1.02 pg) and *Pentamorion* (1.01 pg) represent the lowest values in the study (Table 2). Values within section *Pyxidium* are highly variable, ranging from 1.15 pg for *A. albus* and 2.73 pg for *A. tricolor*, a 2.4 fold range of DNA contents. This is not surprising as Mosyakin and Robertson (1996) report that the section was morphologically and phytogeographically "unsatisfactory," and state that it could probably be further divided into subsections, a conclusion that might be supported by DNA content values.

Accessions of three species (*A. albus, A. blitum, and A. powellii powellii*) were sampled, but DNA contents could not be reliably quantified due to the presence of abnormally large, darkly stained nuclei that were present, side by side with nuclei of normal appearance. These large nuclei are most likely attributable to endopolyploidy, a condition that is frequent in *Chenopodium* and *Amaranthus* seedlings (Bozena Kolano, pers. comm., manuscript in preparation).

To date, differences in genome size have been used to identify hybrid individuals (Trucco et al. 2005; Trucco et al. 2006). In addition to increasing reported DNA content values within *Amaranthus* by eight additional species, we observed that patterns in DNA content might be taxonomically informative both within and among species (Table 2). Additionally we note the contraction of the genome following polyploidization in *A. dubius* and the presence of endopolyploidy in *Amaranthus*.

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